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RESEARCH PAPER

Screening of some indigenous herbal dyes for use in plant histological staining

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Abstract: The efficacies of some indigenous herbal dyes for use in staining plant materials were examined to obtain non-toxic, eco-friendly and cheap stains for use in plant histology. Dye extracts from *Bixa orellana*, *Curcuma domestica*, *Lonchocarpus cyanescens* and *Pterocarpus osun* were used to stain wood sections using the existing standard staining procedures with little modification. All the extracts had affinity for the fibre and vessel elements except the extract from *L. cyanescens*. The extracts from *C. domestica* and *B. orellana* had higher selectivity than those of *P. osun* for fibre. From the results of the absorbance curves, each of the dye extracts from all speciese had minimum of two peaks, indicating that they had two or more colour imparting chromophores except dye extract from *C. domestica*. All the dye extracts were acidic with pH range of 3.77 to 6.77. Therefore, this study shows that dye extracts from *B. orellana*, *C. domestica* and *P. osun* could be solitarily or in combination with artificial dyes for plant histological staining.

Keywords: herbal dyes; indigenous herbs; ecofriendly stains; histological staining; wood sections

Introduction

Forest dwellers depend on plants for tannins, dyes, food as raw materials for a wide variety of traditional products and artifacts (Banderanayake 1999). These products are also relied upon by a great number of people living in the cities. Extracts from plants are used in various fields in Nigeria and other developing countries. They are used as folkloric medicine (e.g. bush medicine used in Herbal remedy), pesticides and insecticides, dyes for textile materials, as well as stains for biological specimens. Plant dyes are utilized locally to dye cloths, domestic fowls, house floor and wall and household utensils. They are also used to preserve animal skin, decorating human body for differentiating animal.

Plants yield many colours for dyeing a variety of articles. Some trees and many herbs produce dyestuffs, and hitherto the bulk of world's dyes can be produced from plant sources. In facts, plants have provided an important source of pigments and tannin for the world (Lillie 1969). Plant dyes are found in the root, root bark, leaves, flowers, stem, stem bark, fruit skins and nutshells. Some plants may have more than one colour or sources of colour among the various parts and/ or at various stages of their growth or development. These dyes are capable of improving the contrast of plant sections, so that distinction can be made between one cell and the other or between cell and its constituents.

However, the applications of synthetic dyes are presently limited because of their hazardous effects on human and animal health. Bhuyan and Saikia (2004) asserted that the prevalent withdrawal from the use of some of these synthetic dyes is very connected with the detection of their hazardous nature. Also, most developing countries can no longer afford the ever increasing cost of synthetic dyes (Avwioro et al. 2005a). These identified problems associated with the use of synthetic dyes now prompted the search for low-cost dyes, especially of biological origin, that will be effective, easy to use, biodegradable and safe to both the human and environment health.

Natural vegetable dyes now gain prominence all over the world, especially in the developing countries due to their eco-friendly nature and hence, their promising usage was used as histopathological stains. This is typified by the most used dye haematoxylin obtained from the Mexican tree, *Haematoxylon campechianum* (Baker et al. 1976). There are the numerous that have examined the potential of natural dyes for use in histopathology (Avwioro et al. 2005a &b; Eom et al. 2001; Padhy et al.

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1990; and Garg et al. 1991). Gaur et al. (1998) also stressed that herbal stains from Saffron, safflower and henna could be successfully utilized for differentiating inactive living and dead nematodes during bioassay and other investigations. Therefore, in the present study, we investigated the efficacies of four herbal dyes in a bid to obtain non-toxic, eco-friendly and cheap stains for use in plant histological studies.

Materials & methods

Collection and preparation of materials

The materials used for extraction of dyes in this work include: matured seeds of *Bixa orellana*, collected at the Polytechnic, Ibadan, Nigeria; dried heartwood of *Pterocarpus osun*, collected in Awosun Village, Ife North Local Government Area, Osun State Nigeria; rhizome of *Curcuma domestica* collected in the Plant Nursery Botany and Microbiology Department University of Ibadan, Nigeria; and the young leaves of *Lonchocarpus cyanescens* collected along Road 1, University of Ibadan, Nigeria.

Ripe, dried fruits of *B. orellana* were harvested. The seeds were shelled from the pods and winnowed to remove the husk, dust and other foreign particles and then kept in polyethylene bag. Matured dried heartwood of *P. osun* was milled into powder using milling machine after thorough cleaning. The powder wood was kept in polyethylene bag.

Fresh matured rhizomes of *C. domestica* was mashed using pestle and mortar after thorough cleaning. The mashed rhizomes were sun-dried to reduce the moisture content before it was kept in polyethylene bag. The young leaves of *L. cyanescens* were collected, rinsed with distilled water to remove dirt. This was air-dried in the Laboratory. The dried leaves were ground using mortar and pestle. The mashed leaves were sun-dried to reduce the moisture content after the sample was kept in polyethylene bag. All the Plant materials used were identified at the Forest Herbarium Ibadan (FHI)

Extraction of the dyes

Dyes were extracted from 1 kg each of matured heartwood of *B. orellana* seeds, *C. domestica* rhizomes, *P. osun* and young leaves of *L. cyanescens* using sohxlet extractor with ethanol as solvent. The dye yield was 205 g for *B. orellana*, 202 g for *P. osun*, 252 g for *C. domestica* and 105 g for *L. cyanescens.*

Staining procedure and slide preparation

The extracted dyes were tested on wood sections of *Cola gigantea* which was collected from the Botanical Garden, University of Ibadan, Nigeria. The plant sections were stained in the extracted dyes for 3 minutes. For simple staining, the sections were washed/rinsed in water before treatment in series of ethanol to remove water molecules (dehydration process) and to remove excess stain (differentiation process in acidified ethanol). The dehydrated and differentiated sections were transferred into absolute xylene in two series to remove last traces of water, to clear the section (making it more transparent) and to remove last traces of ethanol and since xylene is the solvent of the mountant (DPX) used. It prevents cloudiness of the slide and it makes slide to dry fast since xylene is a volatile solvent. The section is mounted on glass slide in DPX mountant.

For the double staining, each section was stained first in dyestuffs extracted. The sections were rinsed in water and then counter stained in Alcian blue. The process of dehydration, differentiation and clearing was done as for the simple staining as explained above and the section mounted in DPX mountant on the slide.

Microscopy

Miscroscopical observation of each slide was made and recorded. Photomicrographs of the slides were made using Olympus photomicroscope with analog camera.

Spectrophotometric analysis

Each of the extracted dyes was crystallized to obtain the solid dye. The 0.0002 g of each sample was dissolved in 10 mL of Absolute ethanol and 10 mL of Absolute ethanol was served as blank. Each of the dye samples was run on UV/ visible spectrophotometers, which automatically subtracted the effect of the blank and plotted the graph of Absorbance against wavelength.

pH determination

The pH of each dye was determined with the use of digital BMF/ pH meter.

Results

Performance of dye extracts on wood sections

The dye extract from *B. orellana* was crystallized easily and imparted its orange colour discriminately on the fibre and vessel members. Though the dye extract from *C. domestica* did not crystallize easily because of the high oil content, it is much more discriminatory in imparting its brilliant yellow colour on fibre and vessel members. The crystal of the dye extract from *L. cyanescens* absorbed moisture readily from the atmosphere and it is *ipso facto* suspected to be rich in sugar content. It did impart no visible colour on any cell. Also, the dye extract from *P. osun* imparted its red colour on all cells but fibre and vessel members took the stain more deeply. Additionally, dye extracts from *B. orellana*, *C. domestica* and *P. osun* are highly specific when used in double staining with Alcian blue. The Alcian blue stained parenchyma and thin wall cells while dye extracts from *B. orellana*, *C. domestica* and *P. osun* stained fibre and vessel members (Fig. 1-3).

pH and absorption characteristics of dye extracts

Table 1 shows the pH values of all the studied plant dye extracts and some selected synthetic dyes. The pH of the dye extracts ranges from 3.77 to 6.77. The result of the absorption spectra of the crude dye extracts and conventional stains are shown in Table 2. A single wavelength of maximum absorption λ_{max} value of 421 and 532 nm is taken from Curcuma domestica dye extract and Safranin O, respectively. The dye extracts, Alcian blue and Phloroglucinol from *B. orellana*, *L. cyanescens*, *P. osun* are also reflected with multiple wavelengths. The recorded wavelengths of the dye extracts within visible region of the electromagnetic spectrum show the presence of colour imparting chromophore in the dye extracts.

Table 1. pH of the dye materials

Dye material	pH
Alcian blue	3.25
Bixa orellana	3.77
Pterocarpus osun	4.21
Phloroglucinol	4.97
Curcuma domestica	6.69
Lonchocarpus cyanescens	6.77
Safranin O	7.61

Table 2. *Electronic absorption spectrum on the crude dye extracts

Dye material	Wavelength (nm)	Absorbance(Gy)
Bixa orellana	327	0.345
	272	0.341
	421	0.326
	430	0.299
	456	0.281
	485	0.230
Curcuma domestica	421	1.148
Lonchocarpus cyanescens	372	0.110
	421	0.107
Pterocarpus osun	474	0.329
	506	0.329
	421	0.190
Alcian blue	372	0.134
	421	0.132
	580	0.204
Phloroglucinol	372	0.131
	421	0.131
Safranin O	532	2.549

Notes: *Values are peak of wavelength and absorbance.

Discussion

Cellular structure differentiation and contrast are enhanced with the selective use of various natural and synthetic stains. There may be needed to apply a combination of these stains in some instances to demonstrate the presence of some tissue structures and/ or cellular inclusions. The ability of a dye to stain specific tissue structures is determined by certain factors. One of factors is the pH of the stain. Acidic structures would be stained by basic dyes while basic structures would be stained by acidic dyes (Avwioro 2002).

Three of the four natural dye extracts from *Pterocarpus osun*, *Bixa orellana* and *Curcuma domestica* distinctively imparted their colours on fibre and other lignified thick wall tissues like the vessels. However, *Curcuma domestica* is more discriminatory than *Bixa orellana* on these tissues. In contrast, the dye extract from *Pterocarpus osun* indiscriminately imparted its colour on almost all cells but fibre, vessel members and other thick wall cells took the stain more deeply.

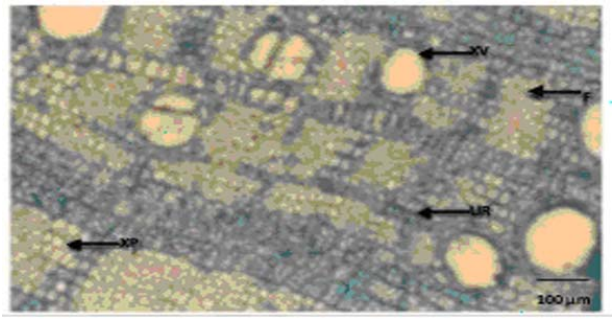

Fig. 1 Transverse section of *Cola gigantea* stem stained with *Bixa orellana* dye extracts & Alcian Blue. F= fibre; UR= uniseriate ray; XP= xylem parenchyma; XV= xylem vessel

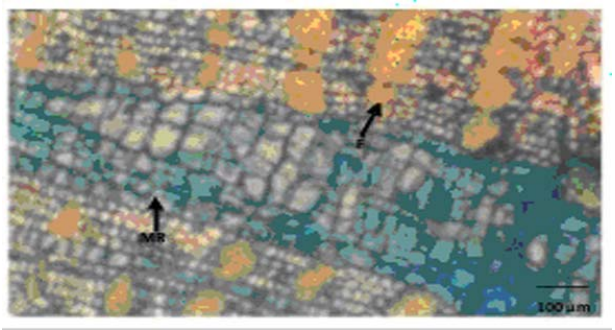

Fig. 2 Transverse section of *Cola gigantea* stem bark stained with *Curcuma domestica* dye extracts & Alcian Blue. F= fibre; MR= multiseriate ray

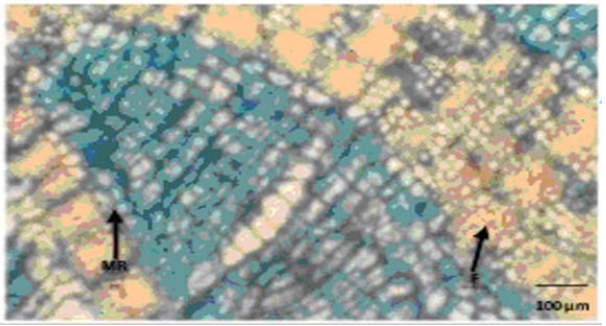

Fig. 3 Transverse section of *Cola gigantea* stem bark stained with *Pterocarpus osun* dye extracts & Alcian Blue. F= fibre; MR= multiseriate ray

The inability of *L. cyanescens* to impart any visible colour shows that *L. cyanescens* cannot be relied upon as a plant histological stain when it was used on wood sections.

Dye extracts from *B. orellana* and *C. domestica* are much more discriminatory in imparting their colours on fibre, vessel members, lignified cells and thick wall cells even without combining with other stain. Dye extract from *C. domestica* proved highly selective because it has inherent affinity for fibre and other lignified cells. *B. orellana* also has a high affinity for fibre and other lignified cells but not as *C. domestica*. When these dye extracts were used in combination with Alcian blue in double staining they become highly discriminatory and specific.

The histochemical behaviour of *P. osun* dye extract and Safranin O is similar because both of them imparted their colours indiscriminately on all cells but fibre, vessel members, other lignified cells and thick wall cells takes stain more deeply. Again both stains become tissue-specific when they are counter-stained with Alcian blue or any other stains that have affinity for thin wall cells. However, *P. osun* extract had pH 4.21 being acidic and Safranin O had pH 7.61 which falls between neutral and alkalinity. Phloroglucinol is a known stain for lignin and is acidic like all the extracted dyes with pH 4.97. Alcian blue is known to have affinity for thin wall cells and is acidic with pH 3.25. Therefore acidity and alkalinity may not be the best parameter to determine the affinity of these dye extracts for tissues/cells.

The absorption spectra of the crude dye extract from *C. domestica*, *P. osun*, *B. orellana* and *L.cyanescens* revealed a range of wavelength of absorption from 327 nm to 506 nm. This falls within the visible region of the electromagnetic spectrum and shows the presence of colour imparting chromophore in the dye extracts. This chromophore may be a polynuclear hydrocarbon compound or a simple aromatic with conjugated side chain. Peak depicts the predominant colour being transmitted by an organic pigment when the complementary colour contained in the light passing through it has been absorbed.

The peak of a colour is determined by the predominant wavelength of absorption in it. Except for *C. domestica* which has only one peak, indicating that *Curcuma domestica* has only one colour imparting chromophore, others have two or more colour imparting chromophores. Popoola et al. (1994) made similar observation of a single peak on *Zingiber officinale*, a member of the same family, *Zingiberaceae*, with *C. domestica*.

The quality of the colour of the extract is a product of the sharpness of its absorption spectrum which in the case of *C. domestica* and *P. osun* is represented by narrow range of the intensity profile. The observation of a narrow absorption band for *C. domestica* and *P. osun* in the spectrum is agreed with the brightness of the dyes in them. This was also observed in *Z. officinale* by Popoola et al. (1994). These two dye extracts imparted their colours brilliantly on fibre and vessel members. *L.cyanescens* dye extract has poor colour imparting feature and this could be attributed to its broad peak and low absorbance. No visible colour was seen on the specimen stained with its dye extract. Dye extract from *B. orellana* shows less broad peak and higher absorbance than that of *L. cyanescens*. *B. orellana* has better colour imparting quality than *L. cyanescens* but not as good in colour impartation as *C. domestica* and *P. osun*.

Conclusions

This study has established the fact that herbal stain from *B. orellana*, *C. domestica* and *P. osun* could be successfully utilized for plant histology. Dye extracts from *B. orellana*, *C.domestica* and *P. osun* are good replacement for Safranin O, Phloroglucinol and any stain with ability to impart its colour on fibre and vessel elements. The results also revealed further that a high performance of these dye extracts is obtainable with the use of ethanol as a solvent for their extraction. This discovery will go a long way in reducing over-dependence on toxic, expensive and non-available exotic stains. Further research should be conducted on the analysis of the active chemical substances in the dye extracts. There is also a need to investigate the potential of the dye extracts in detecting presence or absence of cell inclusions and ergastic substances.

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